3700 Instrument-Spatial Troubleshooting Module

The purpose of this module is to guide the user through troubleshooting a spatial. The software instructions are for Data Collection Software V2.0.

BEFORE PERFORMING ANY TROUBLESHOOTING WORK ON YOUR 3700 INSTRUMENT, PLEASE READ THE ABI PRISM® 3700 DNA ANALYZER USER'S MANUAL FOR SAFETY AND WARRANTY INFORMATION AND FURTHER DETAILS ON USE OF THE SYSTEM.

Please contact technical support if you have any questions regarding the document.

The use of Service Tools is advised only under the recommendation of an Applied Biosystems' representative.

Checking the array for broken capillaries

Before installing a new array it is recommended to check the array for broken capillaries, the loading end, the length of the array and the detection end. Refer to the array diagram for specific areas. If the array is broken, please report the problem to technical support.

Preparing for a Spatial

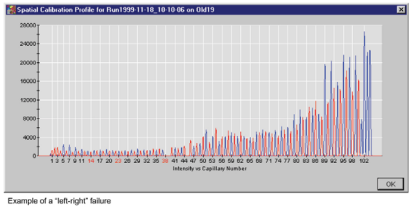
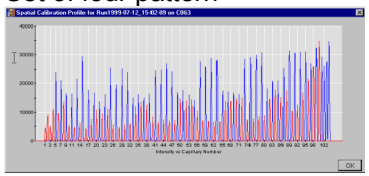
Before starting a spatial it is important to ensure that the instrument is running properly and the reagents are fresh.
Follow the checklist below:

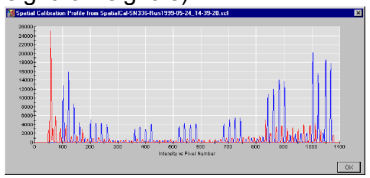
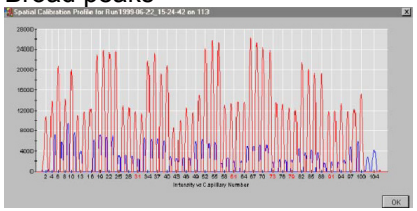
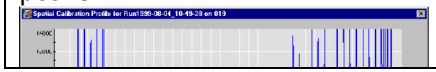
- Perform system maintenance. Refer to the 3700 Instrument Maintenance Module.
- Check for bubbles in the cuvette, if present run the cuvette flush module. Refer to the Checking CCD view module
- Spatial reagents should be fresh. If reagent age is unknown or was stored improperly (recommended storage is 2°-8°C), order a new spatial calibration standard, part number 4325775. The spatial buffer can be made by combining 100uL of 3700 10X buffer and 900uL of fresh HiDi formamide.
- Ensure that the correct run module is being used for the polymer and run type.
  SpatSQ2_POP5DefaultModule for sequencing applications using POP5 polymer
  SpatSQ2_POP6DefaultModule for sequencing applications using POP6 polymer
  SpatGS2_POP6DefaultModule for fragment analysis applications using POP6 polymer
  SpatSNP2_POP5DefaultModule for the SNPShot applications using POP5 polymer

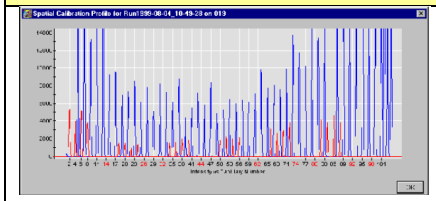
Monitoring a spatial run

During a spatial run, it is possible to monitor the run through the array view page. This page provides a quick overview of a run and is useful in identifying hardware related issues that can affect the spatial.
If a spatial run has failed the spatial information can only be viewed through the entire run display window in Data Collection Software v2.0.

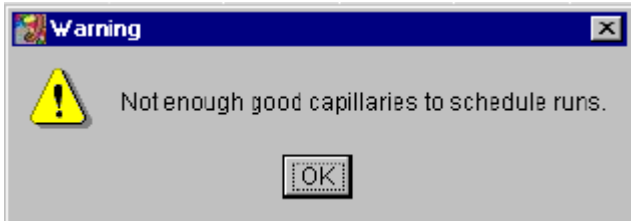
Spatial Passes but quality is questionable. Another spatial will have to be performed after performing the recommended actions.

Observations	Possible Causes	Recommended Actions
Left to Right Variation has a ratio greater than 1:3.  click to enlarge	Bubble in the cuvette	Run the Cuvette Flush module. Refer to the Checking for Bubbles Module and the Checking for Leaks Module if the bubbles in the cuvette persist.
	Cuvette temperature needs optimization	Optimize cuvette temperature in +/- 3°C increments. The temperature can range from 35° to 50°C. Refer to page 4-16 of the ABI Prism® 3700 DNA Analyzer User Guide for instructions on optimizing the temperature.
	Capillary array alignment problem	Verify that the array loading-end header is seated correctly, not under tension, and the capillary tips are positioned in their injection wells.
		Verify that the thumbscrews on the detection end base are not too tight, finger-tight is sufficient, and are screwed on with equal tightness.
	Capillary array damage	Check the loading end of the array for damage and if necessary, remove the array and check the detection end for broken capillaries. If observed, install a new array.
Set of four pattern  click to enlarge Or	Improperly preparing the spatial standard.	Prepare the 200uL of each color standard in a single tube, vortex or pipette-mix, and then pipette 100uL of standard into 2 tubes for running. This ensures that each tube is identical. Spin down samples before placing on the instrument.
	Sample loading issue Bubbles in the sample wells	Spin down samples before placing on the instrument. Inspect the tubes for bubbles in the bottom of the well.
	Bubbles in the sample transfer lines	Verify that the fittings on the ports above the sample transfer syringes are finger tight.

Observations	Possible Causes	Recommended Actions
Pattern of highs and lows (in this case, eight high signals and eight low signals)  click to enlarge	Clogged autoloader tips A non-functioning sample transfer pump	Verify that the sample transfer syringes are tightened to the pump. Finger tight should be sufficient. Verify that the fluid line sinkers are at the bottom of the reservoir. Verify water levels in the water reservoir. Replenish if necessary. Run the Change tips wizard. This will prime the syringes and flush water through the tubing going to the loading tips. During the wizard, monitor both sample transfer syringes for bubbles. If bubbles are present in the syringe but not the tubing from the water reservoir, change the syringe. Replace the autoloader tips. Refer to the change tips wizard for instructions. Refer to the Verifying Syringe Pumps Module for more details.
Broad peaks  click to enlarge	No Sheath Flow	Check the sheath flow syringe for leakage, replace syringe if necessary. Check for leakage around the sheath flow syringe fittings. Clean the area with a lint free tissue moistened with DI water and tighten the fittings until finger tight. Refer to the Checking for Leaks Module. The syringe pump may not be moving. Refer to the Verifying Syringe Pump Module to troubleshoot. Run the Cuvette Flush module. Refer to the Checking for Bubbles Module and the Checking for Leaks Module if the bubbles in the cuvette persist.
Missing peaks 	Improperly mixed red standard	Prepare the 200uL of each color standard in a single tube, vortex or pipette-mix, and then pipette 100uL of standard into 2 tubes for running. This ensures that each tube is identical. Spin down samples before placing on the instrument.

Observations	Possible Causes	Recommended Actions
 click to enlarge	Bubble in one of the red sample tubes	Inspect the tubes with the red standard for bubbles in the bottom, if necessary centrifuge the sample tube.
	Array is not seated properly	Verify that the array loading-end header is seated correctly, not under tension, and the capillary tips are positioned in their injection wells.
	Sample loading issue Bubbles in the lines of one of the sample transfer tips.	Verify that the fittings on the ports above the sample transfer syringes are finger tight.
		Verify that the sample transfer syringes are tightened to the pump. Finger tight should be sufficient.
		Verify that the fluid line sinkers are at the bottom of the reservoir.
	Water lines feeding the sample transfer pumps are not underwater in the water reservoir	Verify water levels in the water reservoir. Replenish if necessary.
		Run the Change tips wizard. This will prime the syringes and flush water through the tubing going to the autoloader tips. During the wizard, monitor both sample transfer syringes for bubbles. If bubbles are present in the syringe but not the tubing from the water reservoir, change the syringe.
	A clogged autoloader tip	Replace the autoloader tips. After installation verify that the tips are tightened until finger tight.
	A non-functioning sample transfer pump	Refer to the Verifying Syringe Pumps Module for more details.
Part of the profile has spiky peaks	Bubbles or particles in cuvette are causing bright events.	Run the Cuvette Flush module. Refer to the Checking for Bubbles Module and the Checking for Leaks Module if the bubbles in the cuvette persist.

The next set of observations is for spatials that generate the following message:



The message can be accompanied by the following errors:

- Red Profile Error
- Blue Profile Error
- Blue and Red Profile Error
- End Caps Blocked
- No Alignment Found
- Non Unique Alignment
- Cap Spacing Out of Range
- Too Many Caps
- No Caps Found

In these cases the spatial fails and does not display a profile in Data Collection V2.0 software. It will be necessary to use the "Entire Run Display" feature to assist with troubleshooting. Another spatial will have to be performed after performing the recommended actions.

Observations	Possible Causes	Recommended Actions
Entire run display is blank Possible error messages: No Caps Found Red Profile Error Blue Profile Error Blue and Red Profile Error	Loss in communication	Restart the system
	Optics	Check the CCD window, for raman lines. Refer to the Checking CCD Window module. If raman lines are not present, contact technical support.
	Sample loading issue The four spatial tubes are incorrectly positioned	Place the four tubes into wells 1–4 of the left 8-bar and spin down samples before placing on the instrument.
	Bubbles in the sample transfer lines	Verify that the fittings on the ports above the sample transfer syringes are finger tight.
		Verify that the sample transfer syringes are tightened to the pump. Finger tight should be sufficient.
	Water lines feeding the sample transfer pumps are not underwater in the water reservoir	Verify that the fluid line sinkers are at the bottom of the reservoir.
		Verify water levels in the water reservoir. Replenish if necessary.
		Run the Change tips wizard. This will prime the syringes and flush water through the tubing going to the loading tips. During the wizard, monitor both sample transfer syringes for bubbles. If bubbles are present in the syringe but not the tubing from the water reservoir, change the syringe.
	Clogged autoloader tips	Replace the autoloader tips. After installation verify that the tips are tightened until finger tight.
	Electrophoresis Issues	Refer to the Electrophoresis Troubleshooting Module
	A non-functioning sample transfer pump	Refer to the Verifying Syringe Pumps Module for more details.

Observations	Possible Causes	Recommended Actions
Entire run display has only one color, blue or red. Possible error messages: Red Profile Error Blue Profile Error Blue and Red Profile Error End Caps Blocked No Alignment Found	Improperly prepared spatial standard.	Prepare the 200uL of each color standard in a single tube, vortex or pipette-mix, and then pipette 100uL of standard into 2 tubes for running. This ensures that each tube is identical. Spin down samples before placing on the instrument.
	Sample loading issue Bubbles in the sample transfer lines	Verify that the fittings on the ports above the sample transfer syringes are finger tight.
		Verify that the sample transfer syringes are tightened to the pump. Finger tight should be sufficient.
	Water lines feeding the sample transfer pumps are not underwater in the water reservoir	Verify that the fluid line sinkers are at the bottom of the reservoir.
		Verify water levels in the water reservoir. Replenish if necessary.
		Run the Change tips wizard. This will prime the syringes and flush water through the tubing going to the autoloader tips. During the wizard, monitor both sample transfer syringes for bubbles. If bubbles are present in the syringe but not the tubing from the water reservoir, change the syringe.
	Clogged autoloader tips	Replace the autoloader tips. After installation verify that the tips are tightened until finger tight.
	A non-functioning sample transfer pump	Refer to the Verifying Syringe Pump Module.
Random capillaries do not have signal present. Possible error messages: End Caps Blocked No Alignment Found	Bubbles in the cuvette prevented fluorescence from some capillaries from being detected	Verify that there is enough polymer in the polymer bottle for the run.
		Run the CuvetteFlush.mod service module. Refer to the Checking the CCD Window Module to determine if bubbles are still in the cuvette.
		Refer to the Checking for Bubbles Module if bubbles remain in system.

Observations	Possible Causes	Recommended Actions
Non Unique Alignment Cap Spacing Out of Range		Replace the inline filter.
	Array is not seated properly	Verify that the array loading-end header is seated correctly, not under tension, and the capillary tips are positioned in their injection wells.
	Broken capillaries on the array	Check the array for broken capillaries at the loading end, the length of the array and the detection end of the array. If breakage is observed, replace the array.
	Capillary Array has dried out and capillaries are blocked with polymer.	Run the Regenerate part of the Change Array wizard to clear the blockage, it may not work depending on the severity of the block.
		Replace with a new capillary array.
	Leaking sheath flow syringe	Inspect the sheath flow syringe for leakage at ports and within the barrel. Replace sheath-flow syringe if leakage is found.
	Leaking inline filter	Inspect the tubing around the inline filter for leakage, indicated by dried polymer. If leaking is observed, replace the inline filter and run the Cuvette Flush module. Refer to the Checking for Bubbles Module and the Checking for Leaks Module if the bubbles in the cuvette persist.
Bright events are present in the cuvette which are detected as false peaks Possible error messages: Non Unique Alignment Cap Spacing Out of Range Too Many Caps	Particles in the cuvette	Inspect the tubing around the inline filter for leakage, indicated by dried polymer. If leaking is observed, replace the inline filter.
		Run the CuvetteFlush.mod service module.
	Capillary array may be misaligned	Verify that the thumbscrews on the detection end base are not too tight and are screwed on with equal tightness.
A few peaks have greater intensity than the others	Bubbles in the cuvette.	Run the Cuvette Flush module. Refer to the Checking for Bubbles Module and the Checking for Leaks Module if the bubbles in the cuvette persist.

Observations	Possible Causes	Recommended Actions
	Presence of reflective particles in the cuvette	Inspect the tubing around the inline filter for leakage, indicated by dried polymer. If leaking is observed, replace the inline filter.
		Run the CuvetteFlush.mod service module.
Capillaries on one side of the array have no fluorescence or A large group of capillaries have a very low signal Possible error messages: Red Profile Error Blue Profile Error Blue and Red Profile Error End Caps Blocked No Alignment Found	Bubbles in the cuvette	Run the Cuvette Flush module. Refer to the Checking for Bubbles Module and the Checking for Leaks Module if the bubbles in the cuvette persist.
	Cuvette temperature needs optimization	Optimize cuvette temperature in +/- 3°C increments. The temperature can range from 35° to 50°C. Refer to page 4-16 of the ABI Prism® 3700 DNA Analyzer User Guide for instructions on optimizing the temperature.
	Array is not seated properly	Verify that the array loading-end header is seated correctly, not under tension, and the capillary tips are positioned in their injection wells.
	Sample loading issue Bubbles in the sample transfer lines	Verify that the fittings on the ports above the sample transfer syringes are finger tight.
		Verify that the sample transfer syringes are tightened to the pump. Finger tight should be sufficient.
	Water lines feeding the sample transfer pumps are not underwater in the water reservoir	Verify that the fluid line sinkers are at the bottom of the reservoir.
		Verify water levels in the water reservoir. Replenish if necessary.
		Run the Change tips wizard. This will prime the syringes and flush water through the tubing going to the autoloader tips. During the wizard, monitor both sample transfer syringes for bubbles. If bubbles are present in the syringe but not the tubing from the water reservoir, change the syringe.
	Clogged autoloader tips	Replace the autoloader tips. After installation verify that the tips are tightened until finger tight.

Observations	Possible Causes	Recommended Actions
	A non-functioning sample transfer pump	Refer to the Verifying Syringe Pump Module.
Wide peaks Possible software messages: No Alignment Found Non Unique Alignment Cap Spacing Out of Range	No sheath flow (the most common cause)	Check the sheath flow syringe for leakage, replace syringe if necessary.
		Check for leakage around the sheath flow syringe fittingsT. Clean the area with a lint free tissue moistened with DI water and tighten the fittings until finger tight. Refer to the Checking for Leaks Module.
		The syringe pump may not be moving. Refer to the Verifying Syringe Pump Module to troubleshoot.

If problem persists, please contact technical support.

Contacting Technical Support

By phone: 1-800-831-6844, option 5
By email: ABTechnicalSupport@appliedbiosystems.com

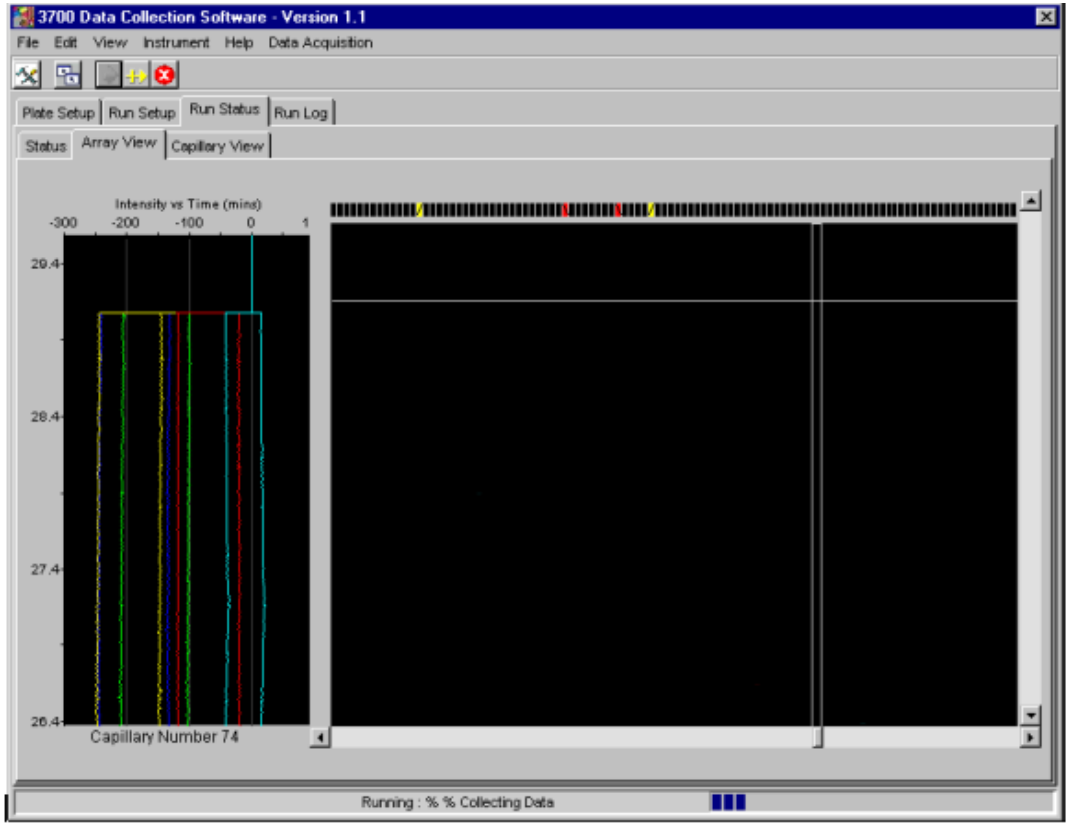
The Array View

The array view is an option in the Data Collection software, which allows monitoring of the data during or after a run. It is provides a quick overview of a run and is useful in identifying hardware related issues that can affect the samples. See the Array View Colors Troubleshooting Module for more information.

How is the array view accessed in Data Collection V2.0?

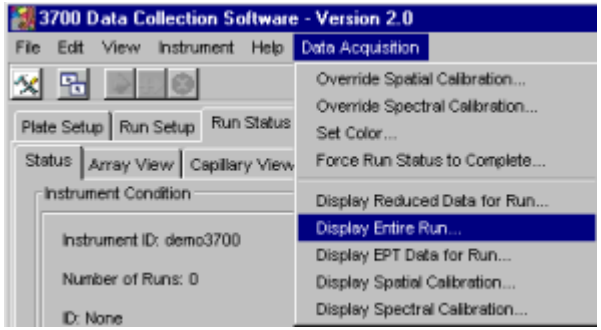
During a run

1) Select the **Run Status** tab > select **Array View.**
   Caution Always exit the Array View when you are finished viewing. Do not leave the window open for extended periods during a run as this may cause unrecoverable screen update problems.

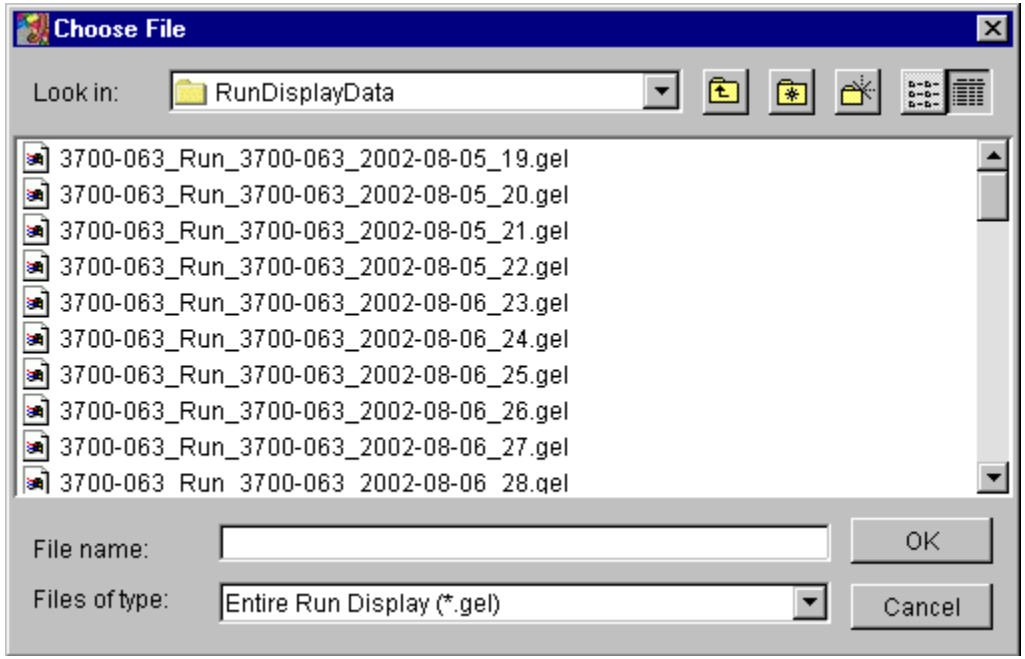


Entire Run Display

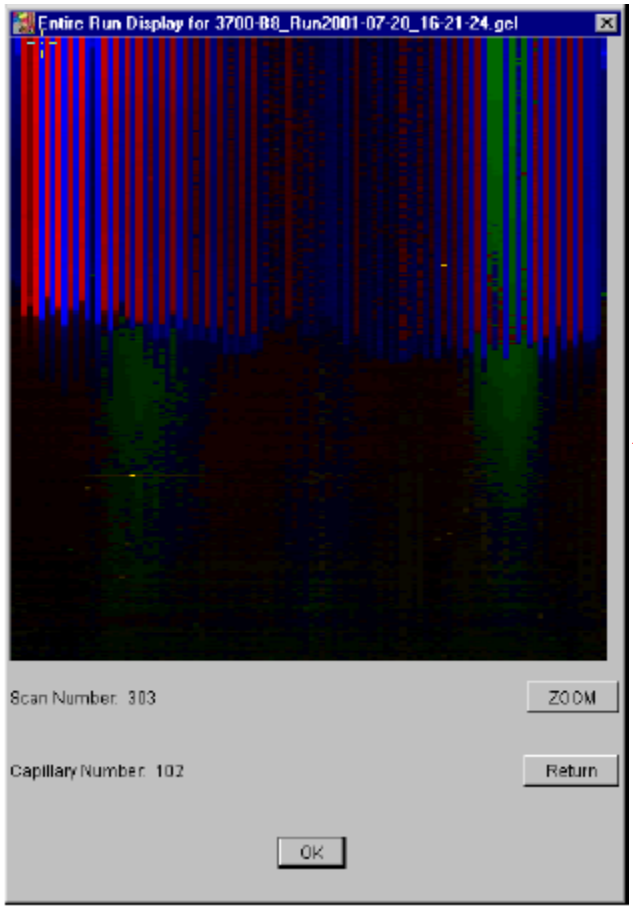
To view the Entire Run Display select the **Data Acquisition** menu >select **Display Entire Run**.


3700 Data Collection Software - Version 2.0
File Edit View Instrument Help Data Acquisition
Override Spatial Calibration...
Override Spectral Calibration...
Set Color...
Force Run Status to Complete...
Display Reduced Data for Run...
Display Entire Run...
Display EPT Data for Run...
Display Spatial Calibration...
Display Spectral Calibration...
Plate Setup Run Setup Run Status
Status Array View Capillary View
Instrument Condition
Instrument ID: demo3700
Number of Runs: 0
ID: None

The **RunDisplayData** folder should be open. Highlight your run of interest and select **OK**.



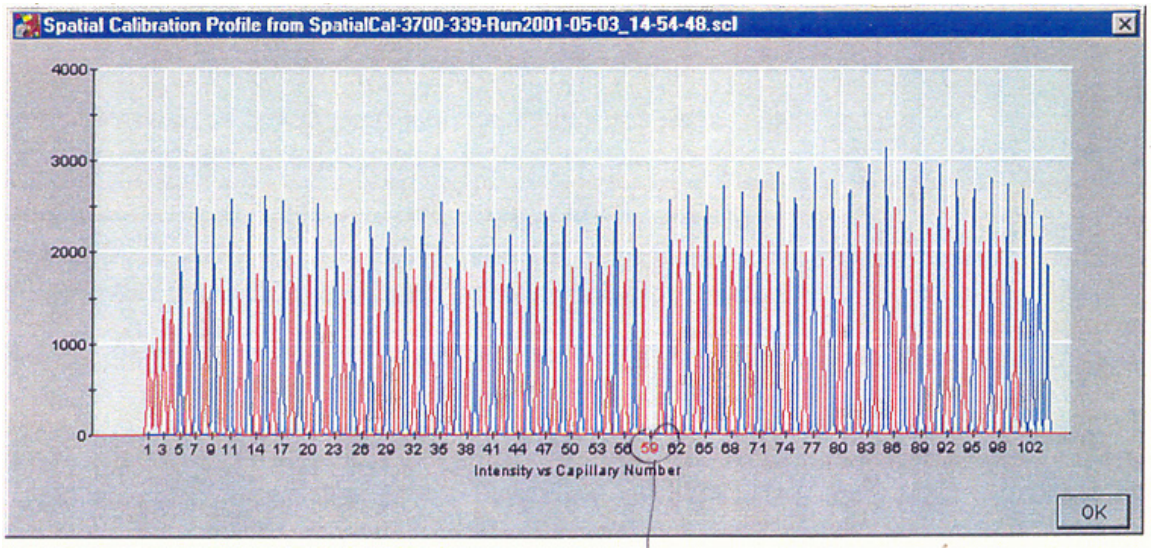
This window will display the entire run.



Two different oligomers, one labeled with dROX (Red) and the other labeled with dR110 (Blue), are loaded to all 104 capillaries in a special pattern.
– Caps 1 - 4: receive red dye
– Caps 5 - 100: receive blue and red, alternating by capillary
– Caps 101 - 104: receive blue dye

Left to Right Variation

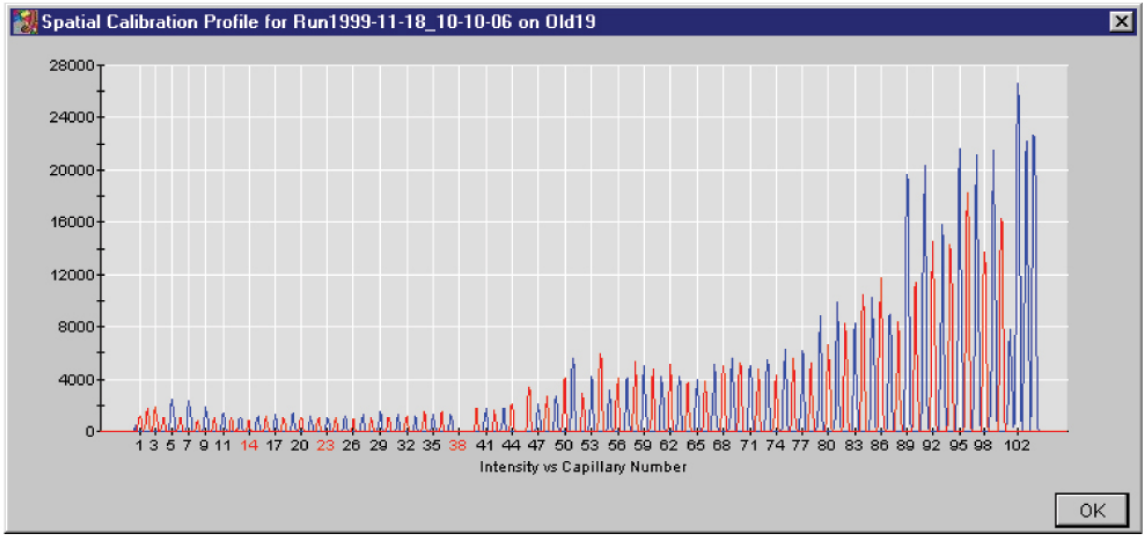
Optimized spatial. Note the uniformity across the array.



Although a peak is missing, the software will extrapolate the position of this capillary.

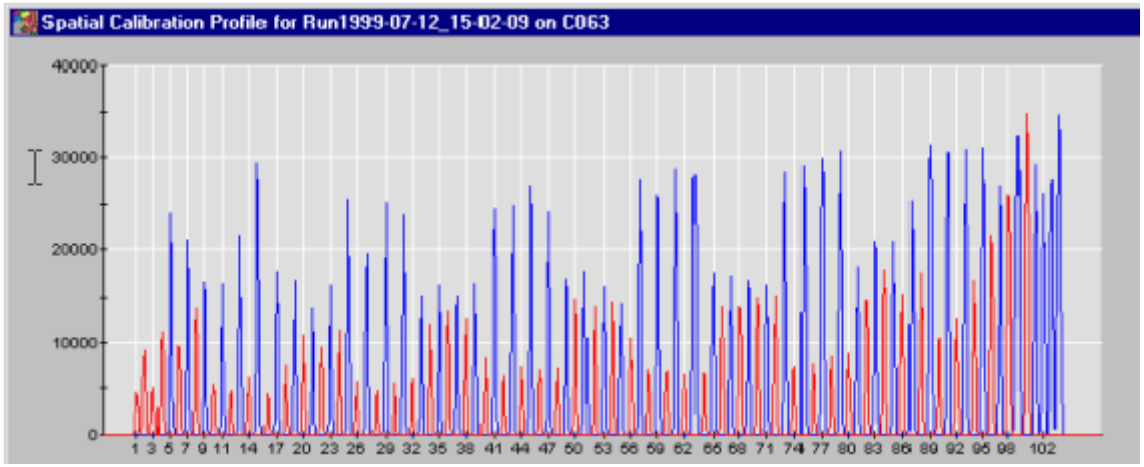
Severe Left to Right Signal Variation

Optimize the cuvette temperature in +/- 3°C increments. Temperature gradients in the cuvette cause deviation of the laser beam. If a bubble is not present in the cuvette, try modifying the method by changing the cuvette temperature in +/- 3°C increments. From the default temperature of 40°C, initially try 37°C and 43°C cuvette temperature when running the Spatial Calibration. Running at both temperatures will help to determine if it is necessary to increase or decrease the temperature incrementally. Continue to change the temperature incrementally to get an optimal spatial. The temperature range should be from 35°C to 50°C, do not go lower than 34°C as the instrument may error. A high ambient temperature can prevent the instrument from reaching temperatures below 34°C. Once the temperature is optimized, run the spatial and all subsequent runs using this array with the optimized temperature. Temperature gradient problems may show other possible profiles such as higher signal on the left than on the right, or a "smile" pattern.

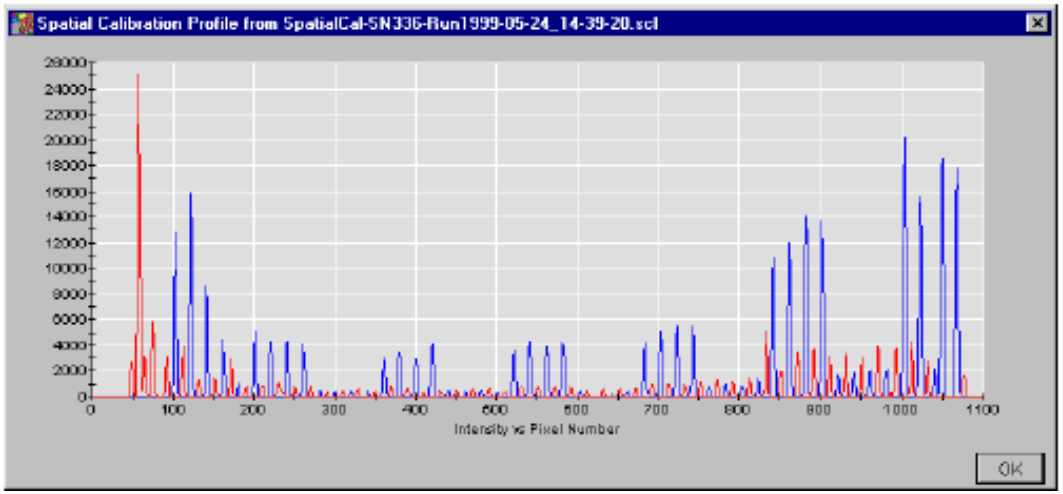


Example of a "left-right" failure

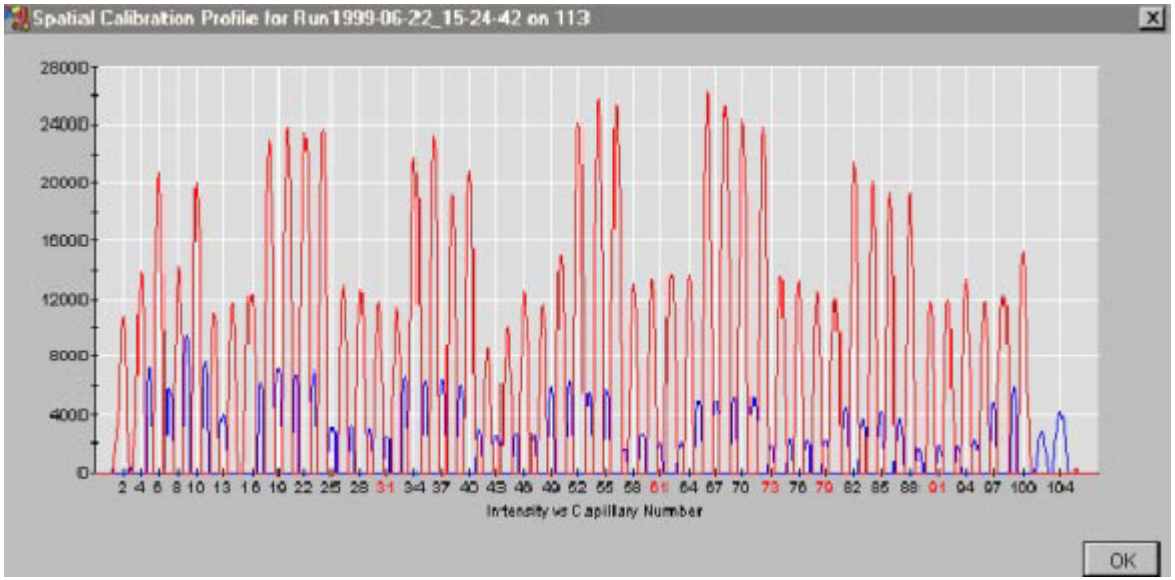
Set of Four



Set of Eight

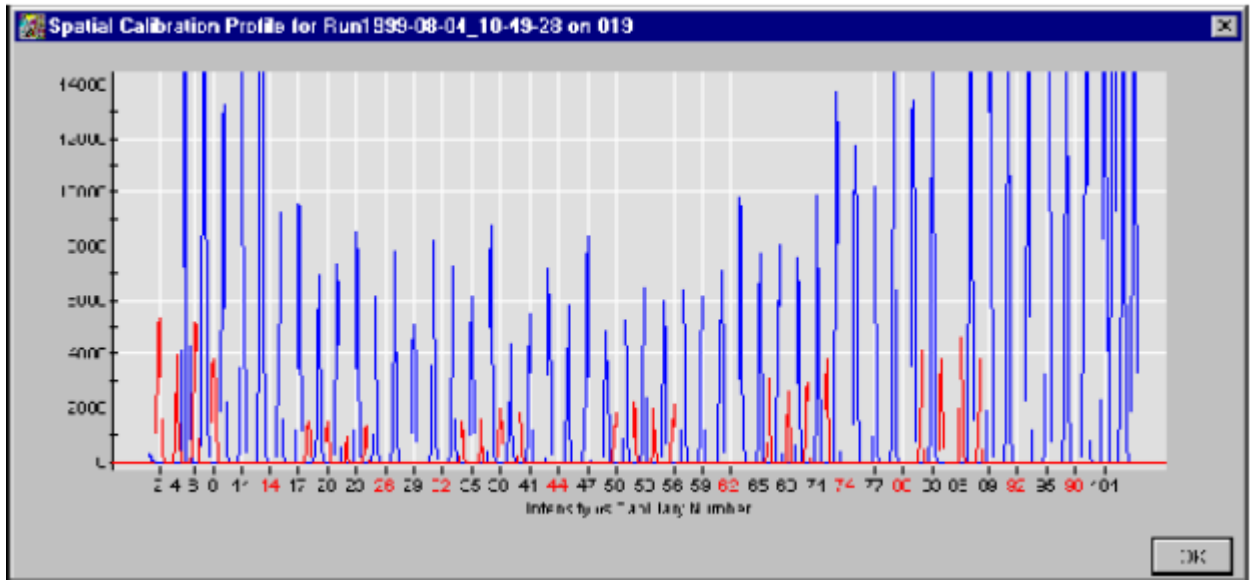


Broad Peaks


Spatial Calibration Profile for Run1999-06-22_15-24-42 on 113
28000
24000
20000
16000
12000
8000
4000
0
2 4 6 8 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82 85 88 91 94 97 100 104
Intensity vs Capillary Number
OK

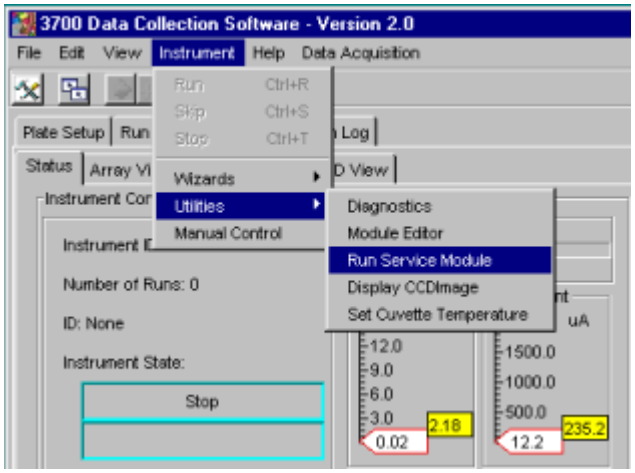
Missing Peaks

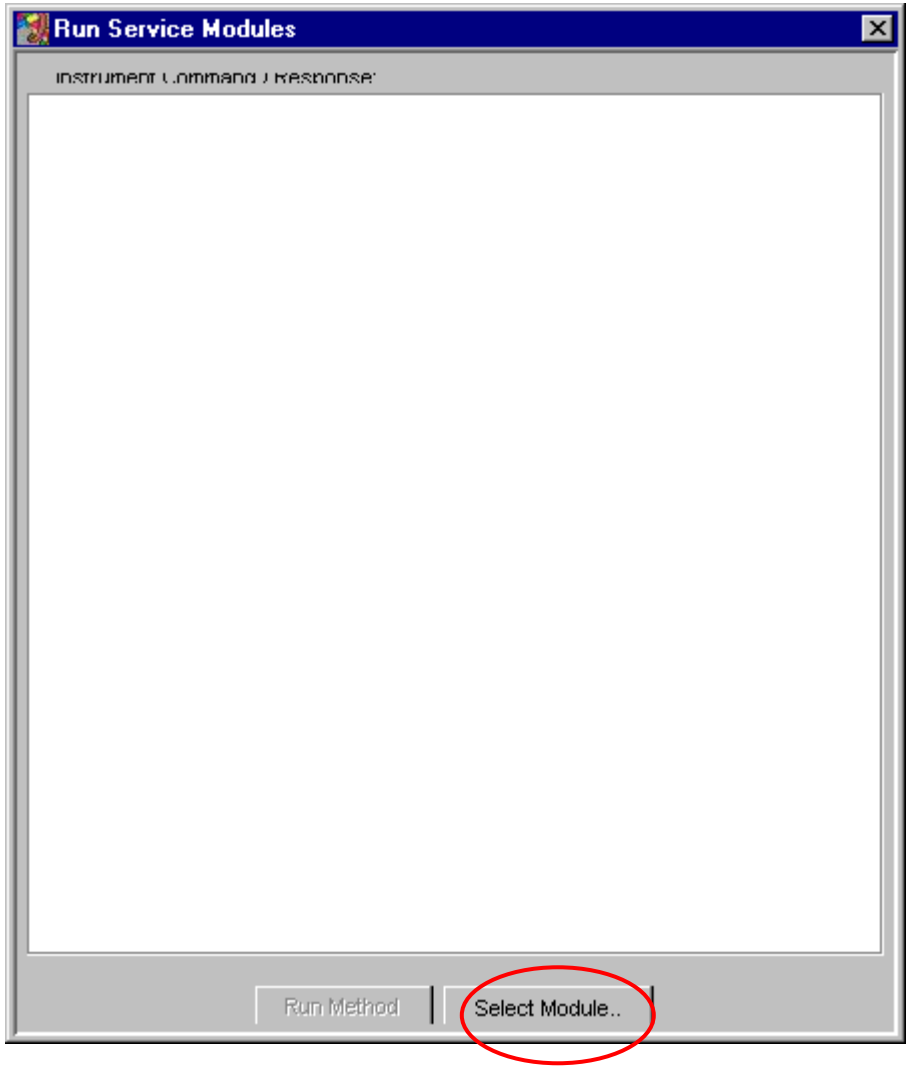
The spatial passed despite missing many red plumes because the spacing was correct and the spatial algorithm could guess the missing plume positions. Although it passed, this profile is indicative of a problem, and the spatial should be rerun.

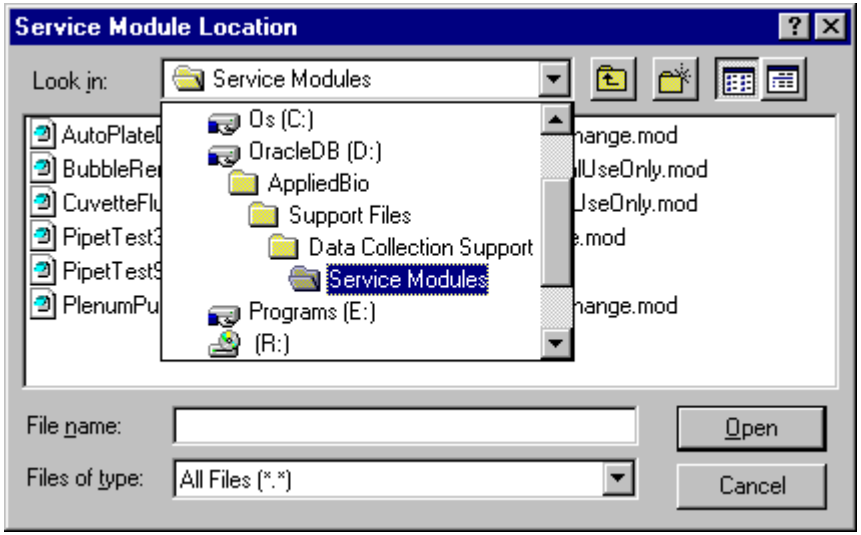


Running service modules within Data Collection V2.0

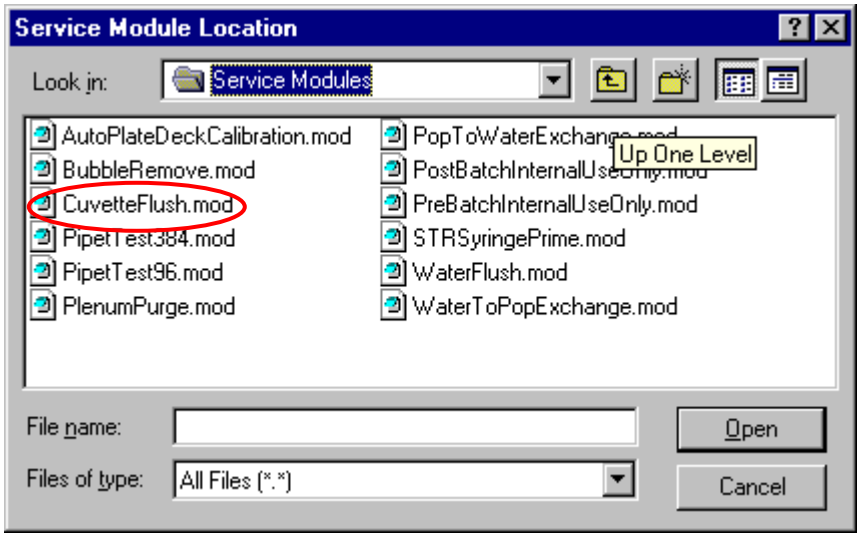
Step 1: Go to the **Instrument** menu > Select **Utilities >** Select **Run Service Module >**
Step 2: Click the **Select Module** button





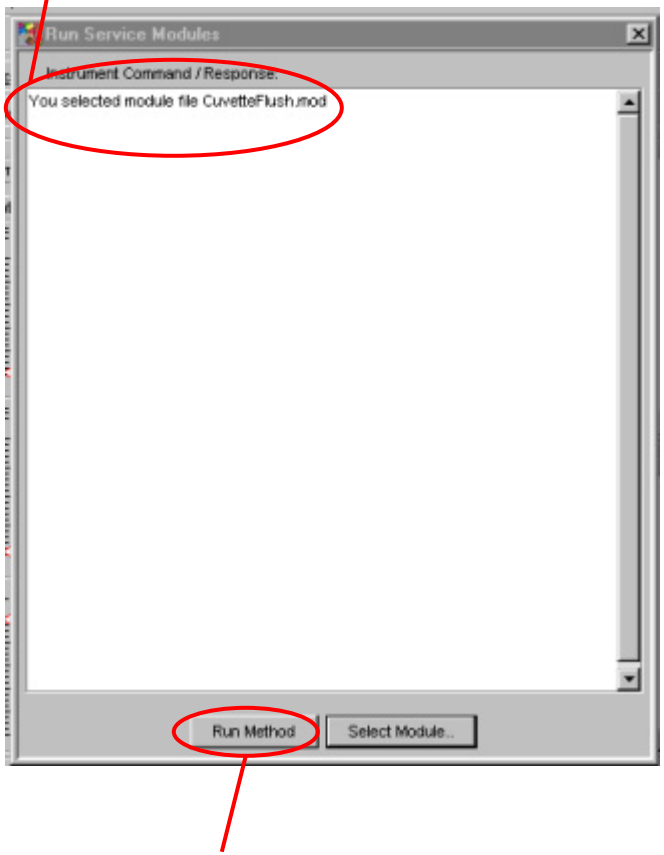


The service module utility should automatically search the Service Modules Folder, if not the folder can be found in the D://AppliedBio/Support Files/Data Collection Support Files/ Service Modules



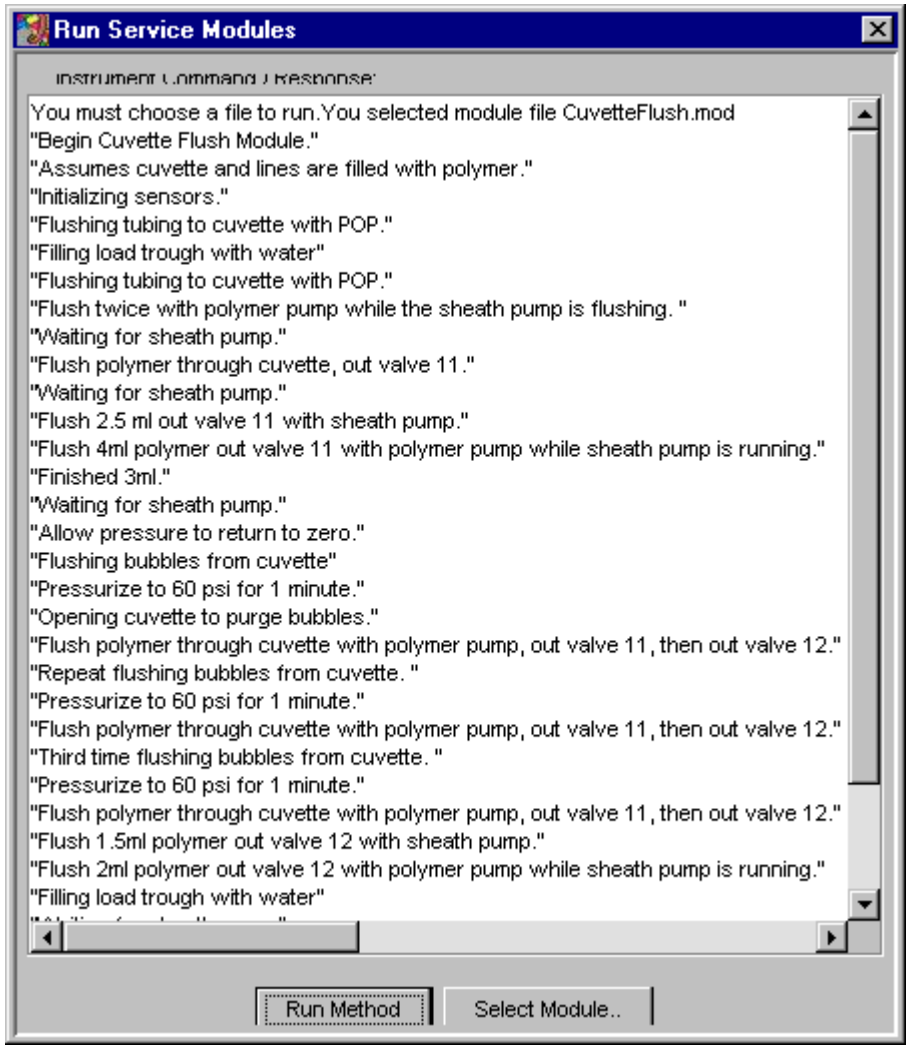
Double click on the module, in this example CuvetteFlush.mod.

The module selected should be listed.



Select Run Method.

After selecting run method, the steps of the service module will be listed.


Run Service Modules
Instrument Command / Response:
You must choose a file to run.You selected module file CuvetteFlush.mod
"Begin Cuvette Flush Module."
"Assumes cuvette and lines are filled with polymer."
"Initializing sensors."
"Flushing tubing to cuvette with POP."
"Filling load trough with water"
"Flushing tubing to cuvette with POP."
"Flush twice with polymer pump while the sheath pump is flushing. "
"Waiting for sheath pump."
"Flush polymer through cuvette, out valve 11."
"Waiting for sheath pump."
"Flush 2.5 ml out valve 11 with sheath pump."
"Flush 4ml polymer out valve 11 with polymer pump while sheath pump is running."
"Finished 3ml."
"Waiting for sheath pump."
"Allow pressure to return to zero."
"Flushing bubbles from cuvette"
"Pressurize to 60 psi for 1 minute."
"Opening cuvette to purge bubbles."
"Flush polymer through cuvette with polymer pump, out valve 11, then out valve 12."
"Repeat flushing bubbles from cuvette. "
"Pressurize to 60 psi for 1 minute."
"Flush polymer through cuvette with polymer pump, out valve 11, then out valve 12."
"Third time flushing bubbles from cuvette. "
"Pressurize to 60 psi for 1 minute."
"Flush polymer through cuvette with polymer pump, out valve 11, then out valve 12."
"Flush 1.5ml polymer out valve 12 with sheath pump."
"Flush 2ml polymer out valve 12 with polymer pump while sheath pump is running."
"Filling load trough with water"
Run Method
Select Module..

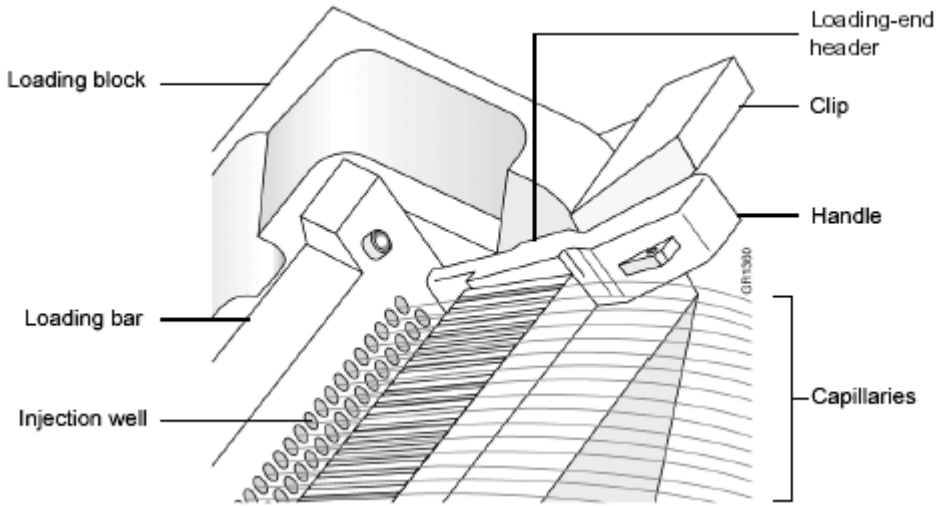
Information
D:\AppliedBio\Support Files\Data Collection Support files\Service Modules\\CuvetteFlush.mod Method Execution Has Successfully Finished.
OK

When this window appears the module has completed, if there is an error during the module, please contact technical support.

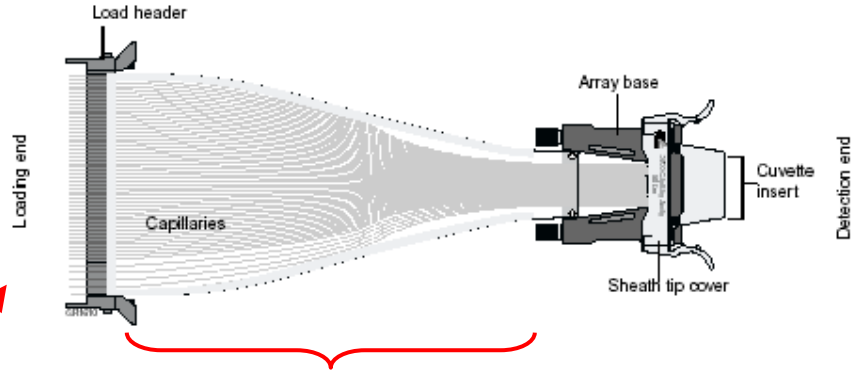
ARRAY

Loading End of Array

The loading-end header is the unit that holds the capillaries at the sample-loading end of the capillary array and aligns them with the injection wells of the loading bar.
The diagram below shows how the loading-end header is held in the loading block.



Array Diagram



Loading end of array

Broken capillaries along the length of the array can be problematic if the array is not changed. Polymer will still flow through the capillary causing buildup of polymer in the area and affecting other capillaries.

Exposing the Detection end of the array

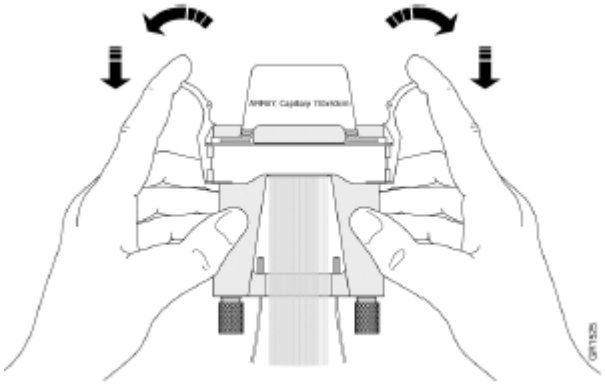
Step 1: While holding the detection-end base in front of you:

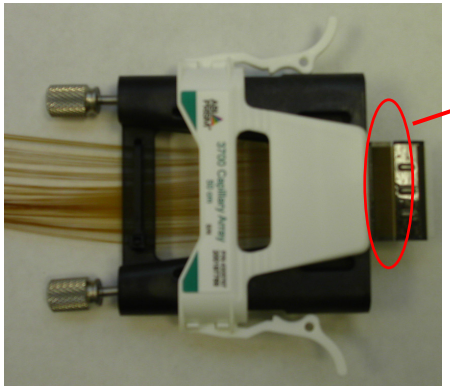
a. Pull the tip-cover clips laterally.

b. Carefully slide the tip cover downwards to expose the tips and O-ring.

IMPORTANT With the cover retracted, the fragile ends of the capillaries are exposed. Any trauma to the tips, such as pressure from fingers during handling, will cause the tips to break and make the array unusable.

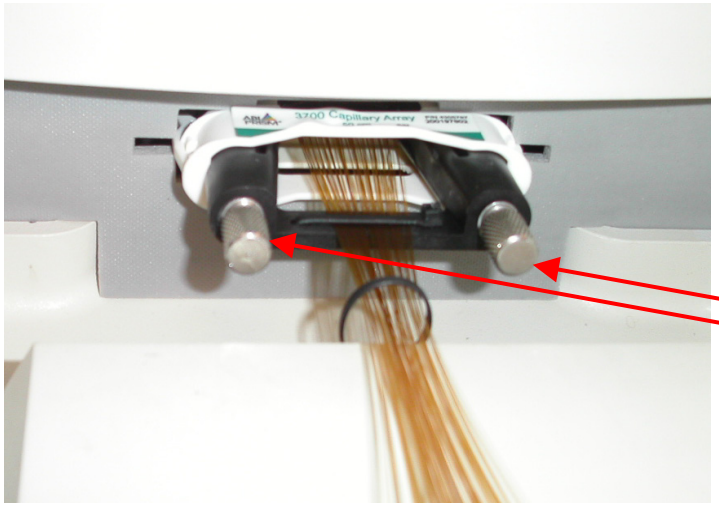
The tips should appear uniform in height. Before installing the array on the instrument, return the tip-cover to its original position.





The capillary tips. Do not touch the capillary tips as they are delicate and may break.

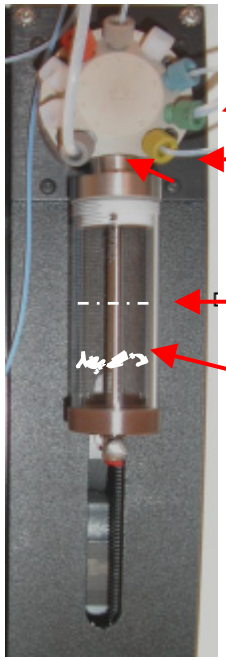
Thumbscrews



Thumbscrews
These should be tightened until finger tight

Sheath Flow syringe and pump

The red arrows identify areas of possible leaks.



Port 8: From polymer bottle to syringe

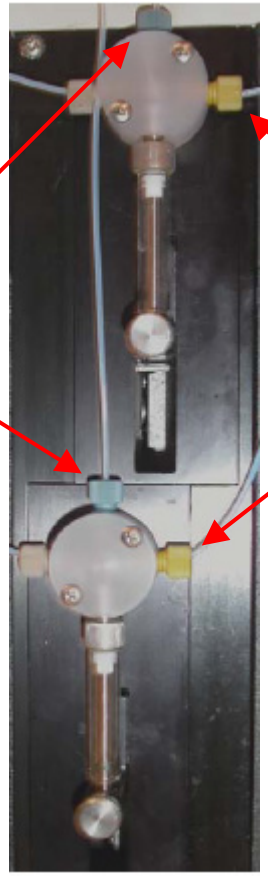
Port 9: From syringe to cuvette

Minimal leakage. Syringe may still be used but should be cleaned regulary and monitored. If problems persists, change syringe.

Severe leakage. Replace syringe.

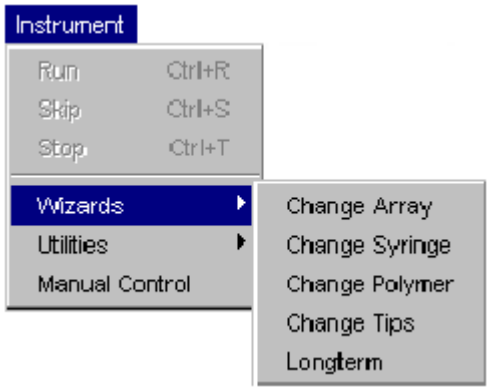
Dried polymer appears as a white residue inside of the barrel. If present the dried polymer acts as an abrasive against the plunger and can damage the seal to the glass barrel. If the seal is damaged, bubbles can be introduced at this stage. Replace the syringe using the Change syringe wizard.

Sample Transfer Syringe

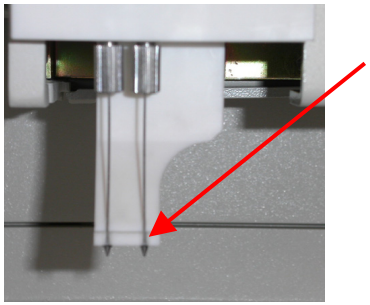


Running the wizard

Step 1: Select Instrument
Step 2: Select Wizards
Step 3: Select the appropriate wizard.

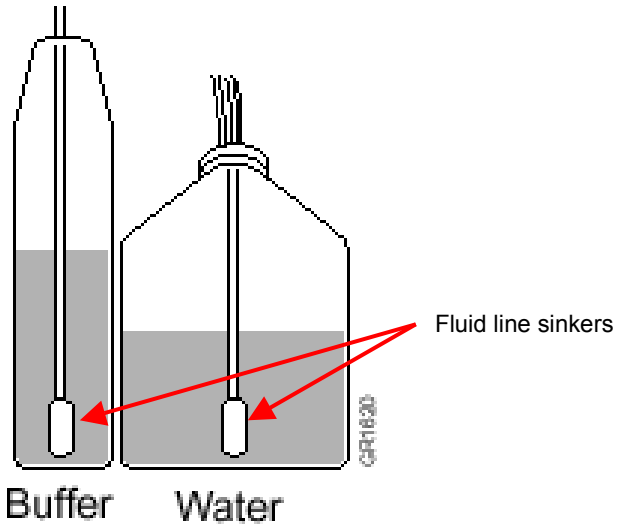


Autoloader Tips

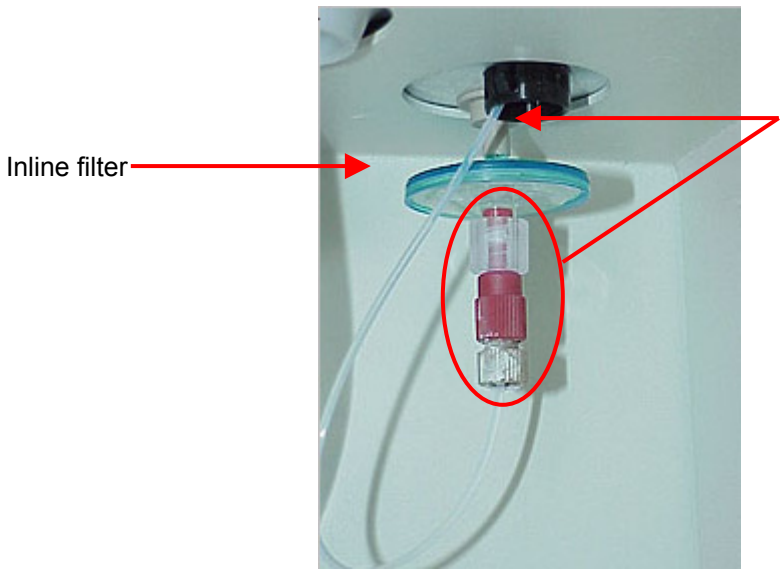


Autoloader tips
Tips should be wiped in a downward direction to prevent clogging during cleaning.

Fluid Line Sinkers



Inline Filter



Restarting the 3700 DNA Analyzer

IMPORTANT To prevent firmware and software memory problems, we recommend that you restart the instrument and the software once a week.

To shut down and restart the instrument:

Step 1: The instrument should not be running or extracting data.

Step 2: Close the 3700 Data Collection software by selecting **Shutdown** from the **File** menu.

Note You cannot use the Close button to exit the software.

Step 3: Close the OrbixWeb Daemon software by right-clicking on its button in the taskbar and selecting **Close** from the pop-up menu. If you get a run-time message, click **OK** to close the message.

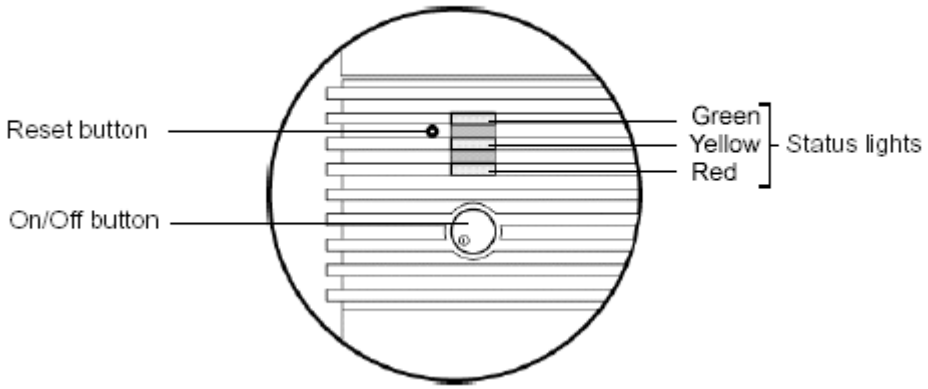
IMPORTANT Do not shut down the OrbixWeb™ Daemon until after you have shut down the Data Collection program.

Step 4: Restart the computer. After logging in**,** OrbixWeb™ Daemon should have automatically launched. **Do not** start Data Collection at this time.

Step 5:

a. Turn off the instrument using the On/Off button.

The following diagram shows the left front grill.



b. Wait 30 seconds.

c. Turn on the instrument.

Step 6: When the green status light is steady, wait 1 minute.

Step 7: Restart the 3700 Data Collection software.